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abbreviations: NA - neutravidin agaose UL - streptavidin ultralink

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collection round double round (selection round without amplification with RNA from CR)

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abbreviations:

NA - neu
UL - stre

neutravidin agaose streptavidin ultralink

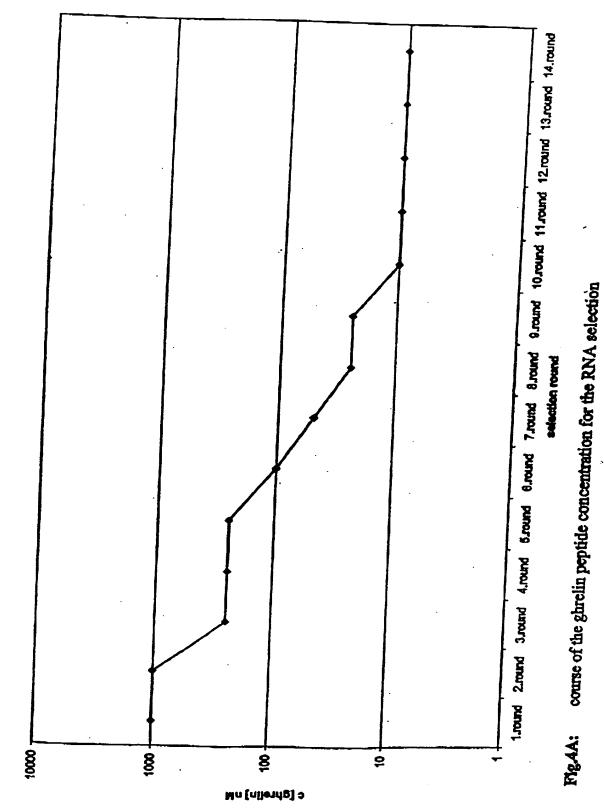
collection round double round (selection round without amplification with RNA from CR) 88

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RNA 6nmol 1mmol 500pmol 1000pmol 2°F-RNA 3mmol 1nmol 500pmol 1000pmol 1000pmol Fig 3: amount of RNA/2°F-RNA used in the selection process

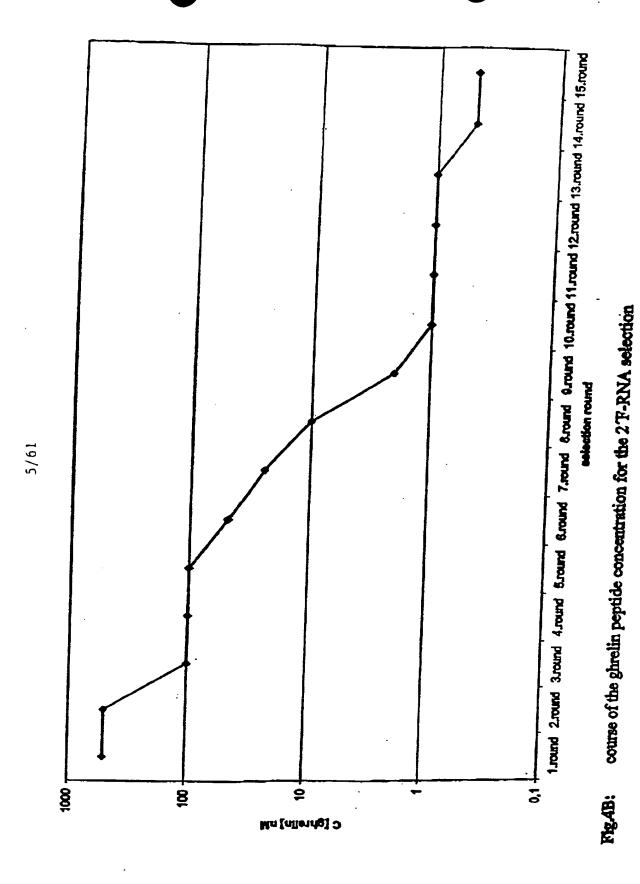
3/61

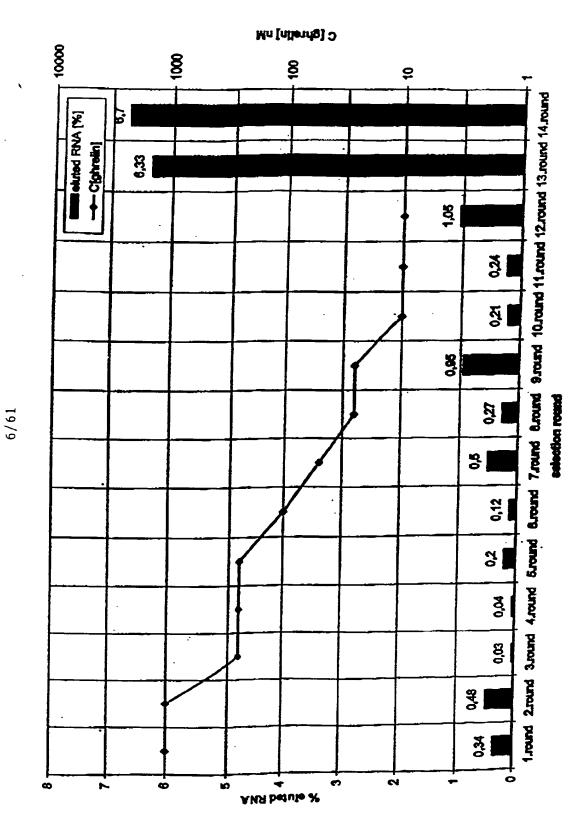
Ffg. 3



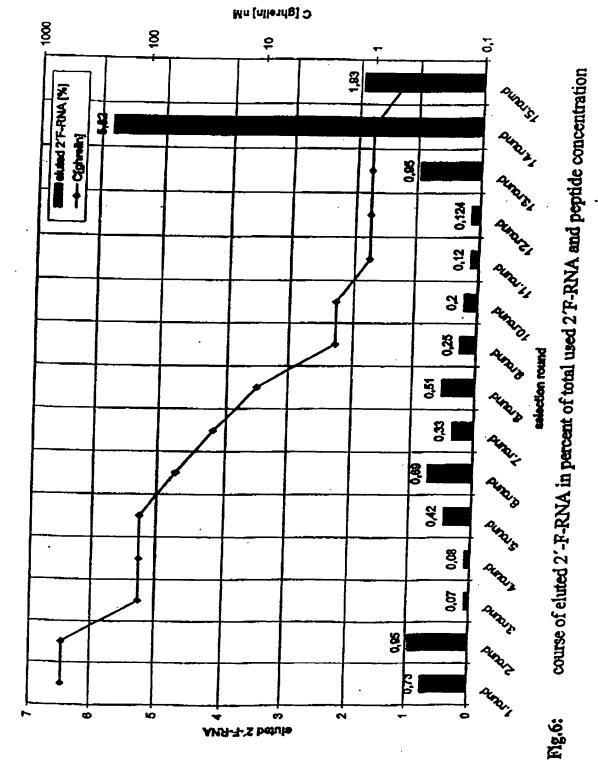
19/5







Course of cluted RNA in percent of total used RNA and peptide concentration Fig. 5:



7/61

F19. 7A

TOCT 10T	9		4						
binding	38		hindled	3 K		test for	E	DR	
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12	2	12	12	:	3				
			2	2	2	14	7	14	
	31.6				1				
206			T						
	1			14,5		53.8			
4,98			15.6			T		1	
0.78			70	1	1	1	23.3		
0.15		T		1	1	282			
0.13	1	1.	Ĭ			6,6	·		
CY S		1,05	1,55		6.33*	35	T	18	
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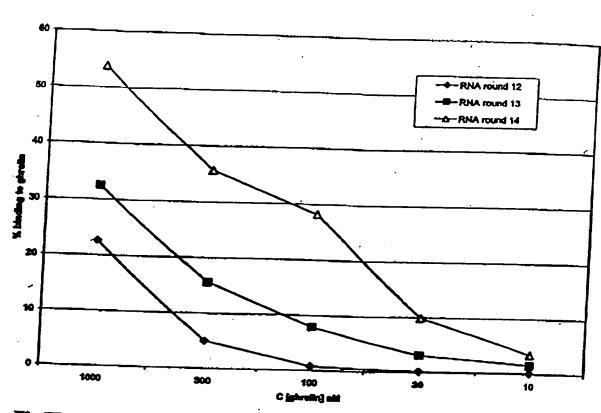


Fig.7B: Improvement of the RNA pool binding to D-ghrelin monitored over the double rounds

F19. 84

														F-RNA
DR (3)	,	2											5,75	1,93* for the 2
CR (3) DR (3)	16	3					19.6							nd 13 to 15
test for binding	15					2 3	32,7	265	101	101	7,1	14		d from rour
]	14											180	700	o.75 erforme
CR(2) DR (2)	41					200	7697							assays p
test for binding	14					AK	2	35,2	29	9.6	30	3		Fig.8A Tab4: double rounds and binding assays performed from round 13 to 15 for the 2 F-RNA selection; data in percent binding to D-ghrelin, * sequenced
(E)	13											0.95	5	ding to
<u> </u>	13				31.4									ouble re
test for binding	. 13				42,4	28.8		C'01	8,49	3,76	0,72			A: de
	round		ပ	[ghrelin]	3µM	lμΜ	200-16	MINOC	100mM	30nM	10nM	InM	SOOM	Fig.8A Tab4: selection; data

10/01



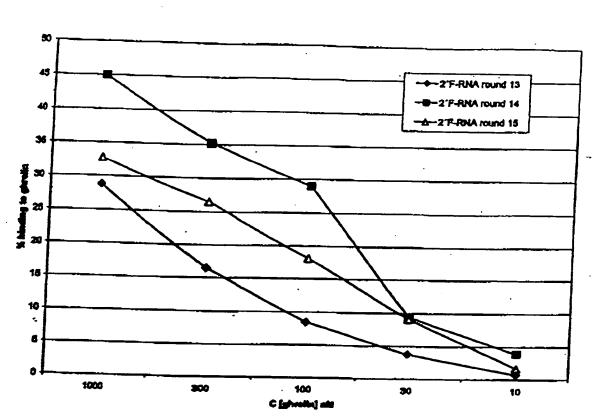


Fig.8B: Improvement of the 2 T-RNA pool binding to D-ghrelin monitored over the double rounds

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26-JAN-05 14:21;

12/61

Automated in vitro-Selection against Rat p-Ghrelin

Round No.	A	В	C	Vold	Remarks
1	1 µM				manual round
2	1 μΜ		·		manual round
3	5 W	10 W	15 W	void 5 W	wash volume: 90 µl per wash (W) [D-Ghrelin] = 500 nM
4	δW	10 W	15 W	void 5 W	48
5 .	500 nM	167 nM	56 nM	. void	15 washes from round 5
6	500 nM	167 nM	56 nM	void	
7	500 nM	167 nM	66 nM	void	
8	500 nM	167 nM	56 nM	void	
9	167 nM	58 nM	19 nM	vold	
10	187 nM	56 nM	19 nM	void	
11	167 nM	56 nM 1	9 nM	void	
12	56 nM	19 nM 6	2 nM	vold	
13	56 nM	19 n) (6	2 nM	. vold	
14	ı	19 pM 6.	2 nM	void	
15 16	<u> </u>	- 	2 nM	void	
17	l	 	1 nM	void	·
18			I nM nM	void void	
19	 		nM	void void	

Fig. 9

Worksurface of the Robot for Automated in vitro-Selection of RNA

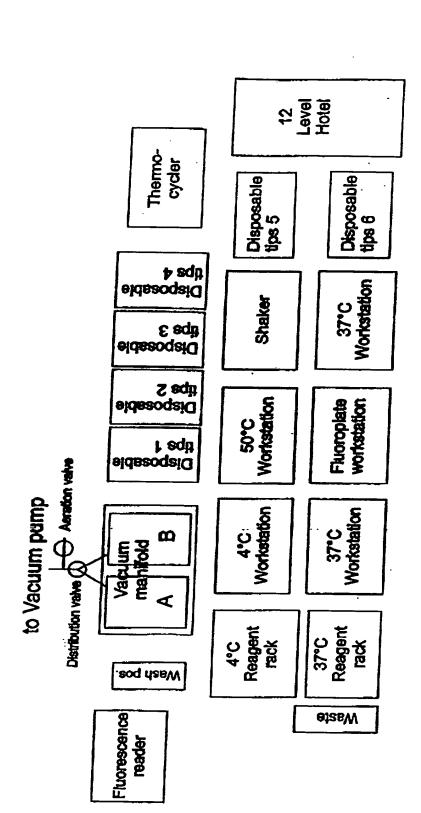


Fig. 10

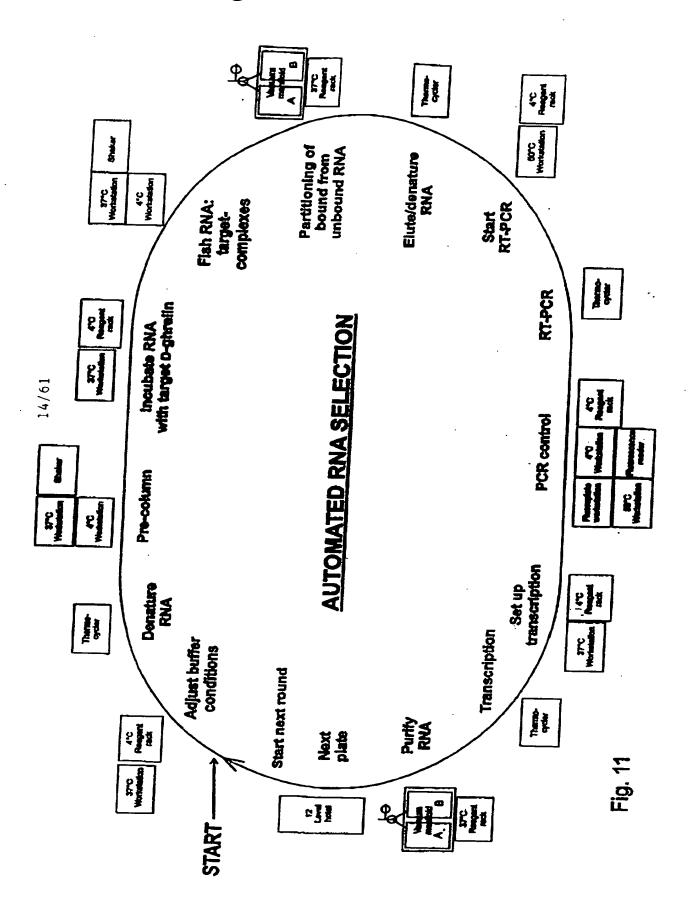
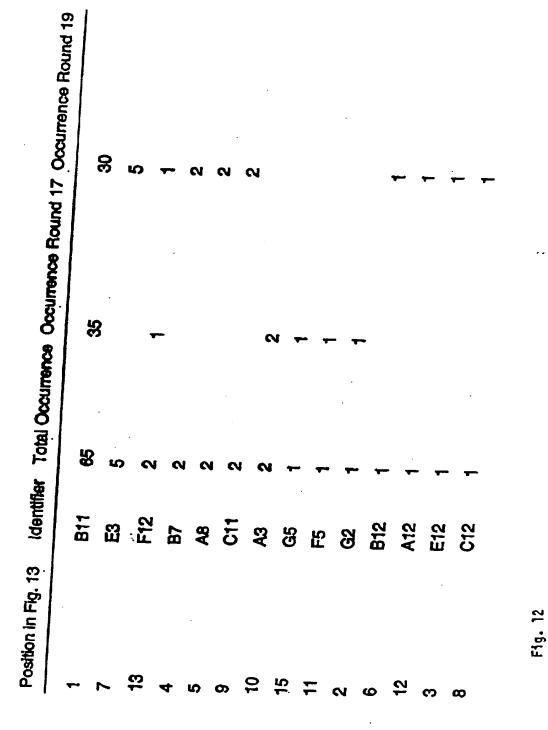


Table 5



DE.40R-Primer (rev. und

compl.)

CGUACCACUGUCGGUUCCAC

E12 B12 C12 CLJ A12 2 E3

GGAGCUCAGACUCACUCGGGGGG---AGGGGAGGAAAAA-----AAAAC--GUAAAUCCGAAGGGAACCAADCCGAC--ACGUACCACUGUCGGUUCCAC GGAGCUCAGACUUCACUCGOGOGO -- AGGCAAUA -----AAAACU -- OUAAGUCCGAAAGGUAACCAAUCCUAC --ACGUACCACUGUCGGUUCCAC

GGAGCUCAGACUUCACU CGUGUG---AGGUAGUAAAAAAA---AAAAC--GUAAATCCGAAGGUAACCAAUCCUAC-ACGUACCACUCGGUUCCAC

GGAGCUCAGACTUCACTUCG DR. 40P-Primer Primer moieties underlined and in bold

GGAGCUCAGACUUCACUCGUGUG---AGGCAAU-----AAAACG--UAAGUCCGAAGGUAACCAAUCCUAC--ACGUACCACUGUCGGUUCCAC A8 GGAGCUCAGACTUCACUCGUGUG---AGGCAAU------AAAACA--UAAGUCCGAAGGUAACCAAUCCUAC--ACGUACCACUGUCGGUUCCAC

GGAGCUCAGACUUCACUCGUGUG---AGGCAAU-----AAAACU--UAAGUCCGAAAGGUAACCAAUCCUGC--ACGUACCACUGUCGGUUCCAC

GGAGCUCAGUCACUCGOGOGO ---AGGCAAU-----AAAACOOGOAAGOCCGAAGGOAACCAAOCCOAC--ACGUACCACUGUCGGUUCCAC

GGAGCUCAGACUUCACUCGUGUG---AGGCAAU-----AAAACU--UAAGUCCGAAGGUAACCAAUCCUAC--ACGUACCACUGUCGGUUCCAC GGAGCUCAGACUUCACUCGUGUG---AGGCAGV-----AAAACV--UAAGUCCGAAGGUAACCAAUCCUAC--ACGUACCACUGUCCGUUCCAC

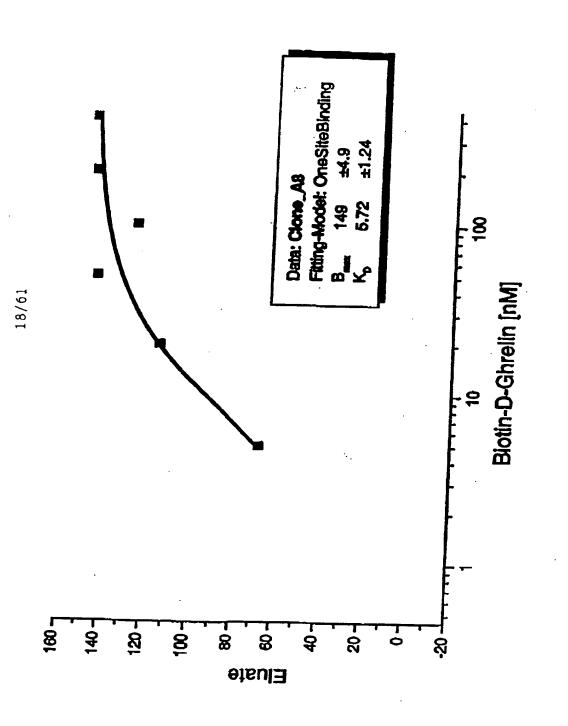
Sequences of the (+) strand

complete forms

core forms:

B11 G2 B12 B12 B12 B12 C11 C11 C11 R5 R12 G5





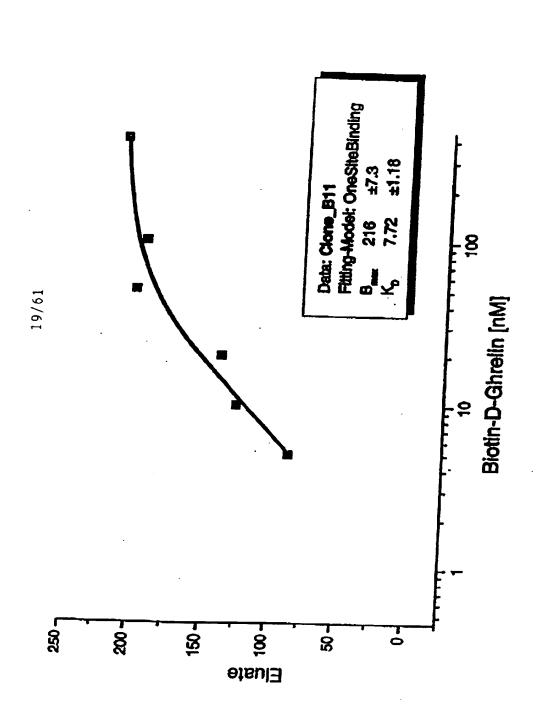
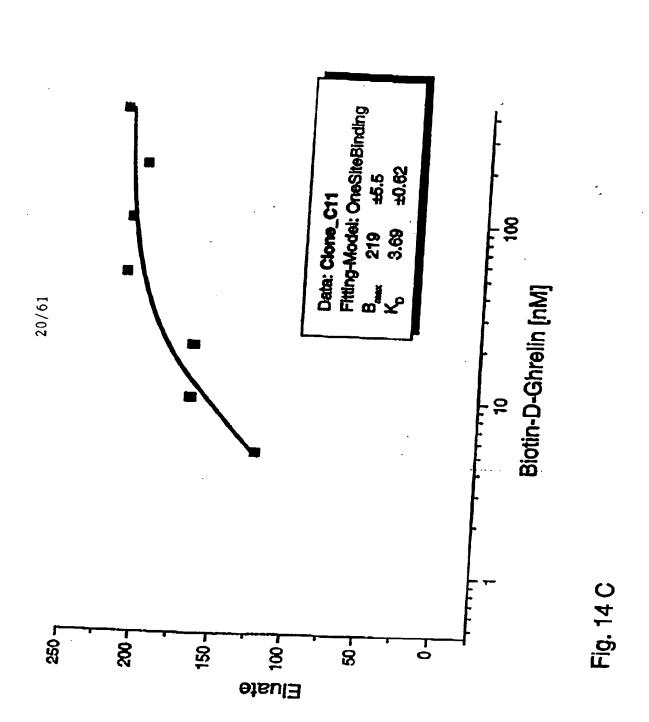
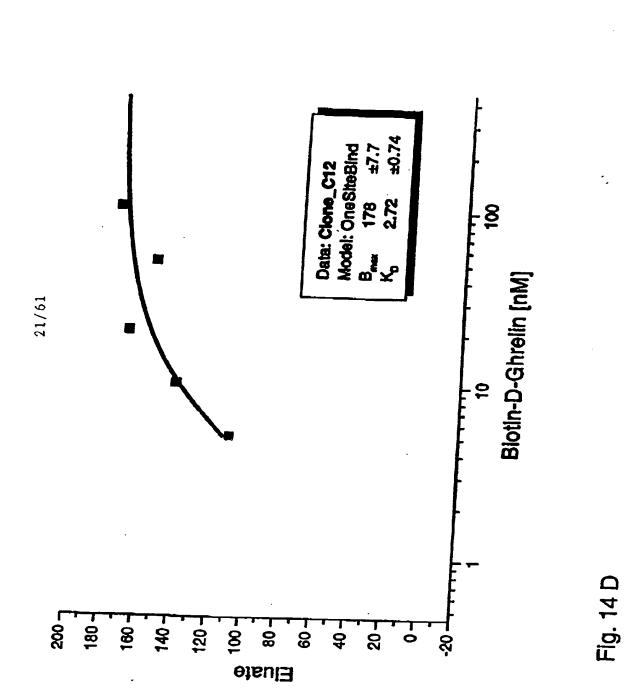


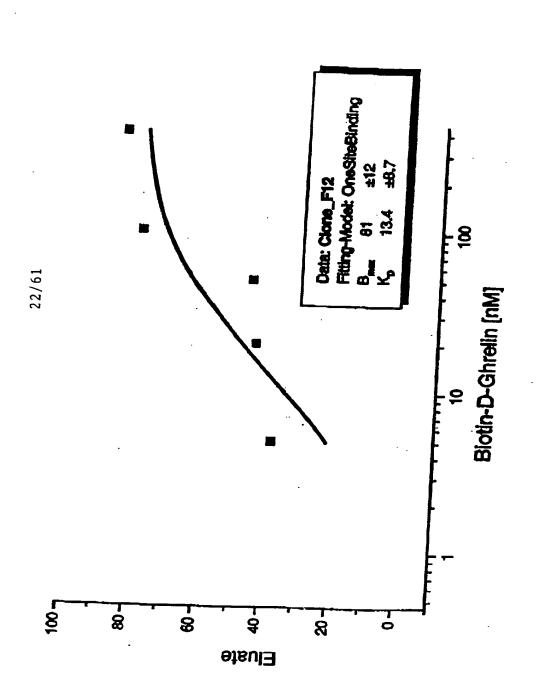
Fig. 14 B











ig. 14 E



Table 6

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Clone B11

[D-Ghrelin] in nM	% RNA bound
0	0
3	. 2
10 30	8
100	35 62
300 1000	76
3000	75 8 3
	6 3

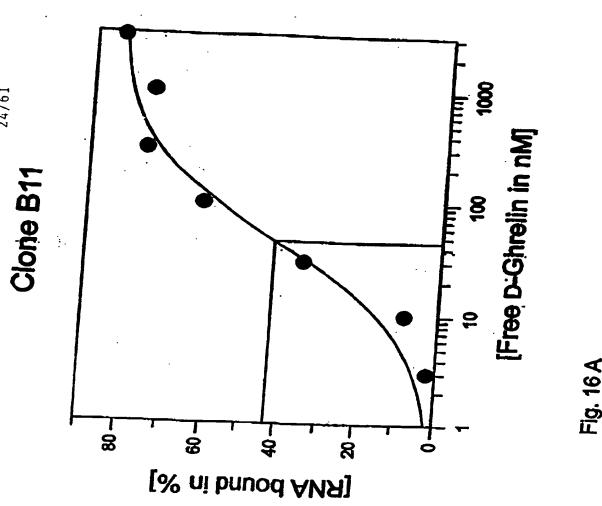
Clone F12

[D-Ghrelin] in nM	% RNA bound
0	0
3	3
10	10
30	29
100	48
300	64
1000	91
3000	8 8

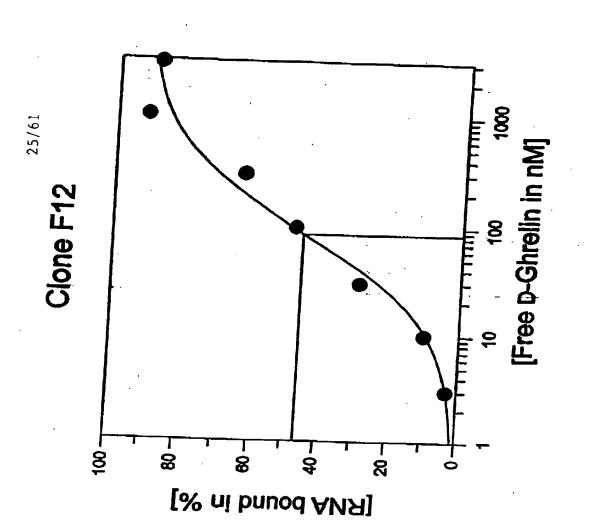
Clone E3

[D-Ghrelin] in nM	% RNA bound
0	0
3	1
10	5
30	20
100	54
300	65
1000	89
3000	85





Clone B11 Activity 8 K, value 4



Clone F12 Activity 91.9 ± 4.1% K, value 87 ± 16.8 nM

Fig. 16 B

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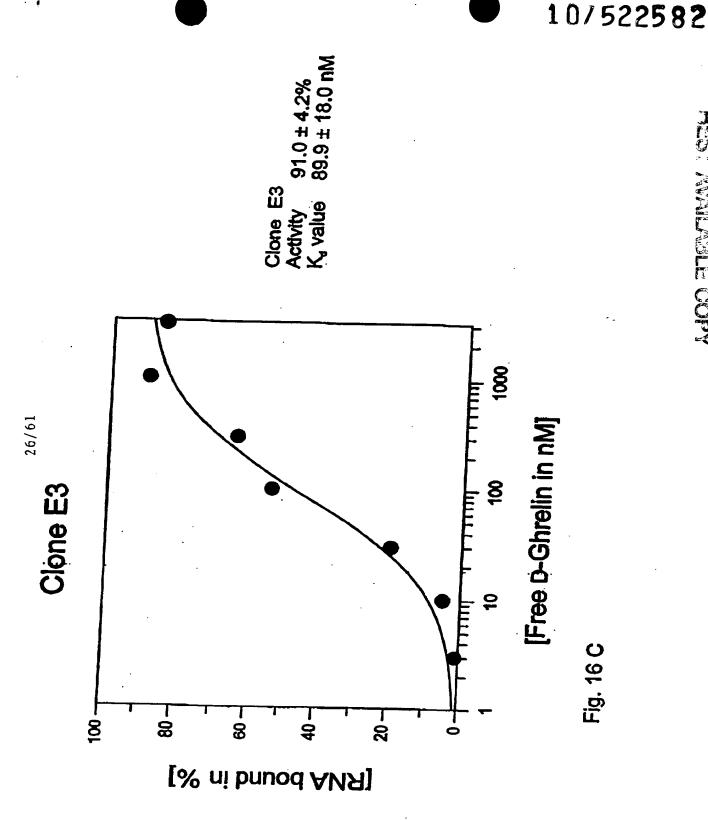
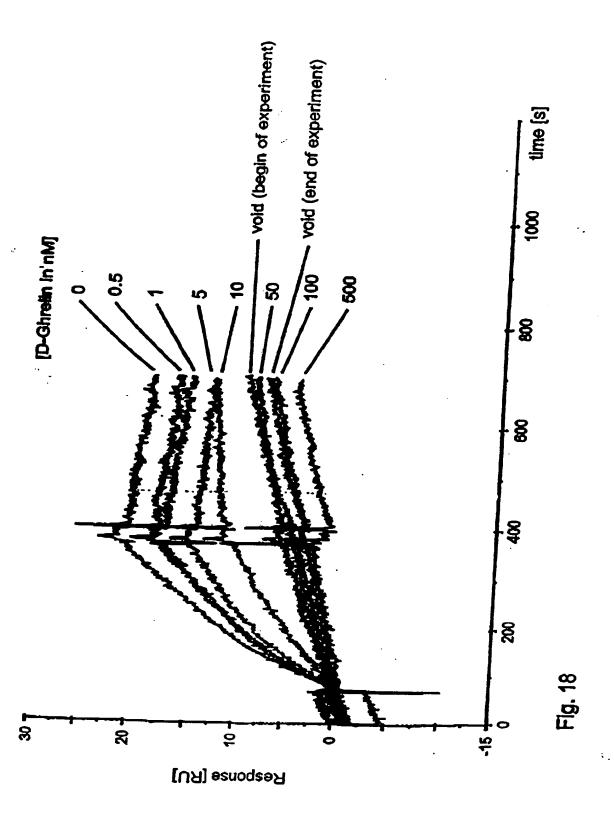


Table 7

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Clone	K _D [nM]
A3	203
A8	98
A12	237
B7	139
B11	205
B12	135
C11	135
Ç12	17
E3	227
12 5	171
12	142
2	111
5	207
	164

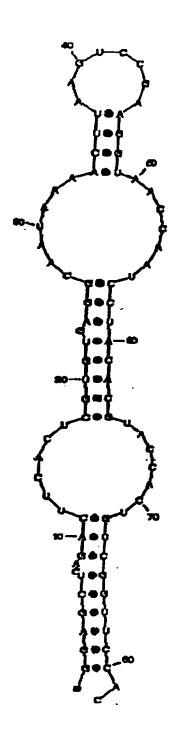
Fig. 17





Clone B11





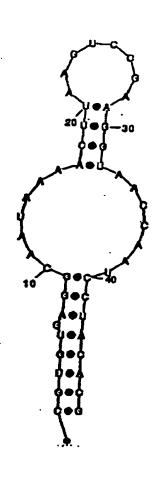


Fig. 19

Table 8

Clone	K _o [nM]
D-B11	206
L-B11 trunc.	104.
D-B11 trunc.	122

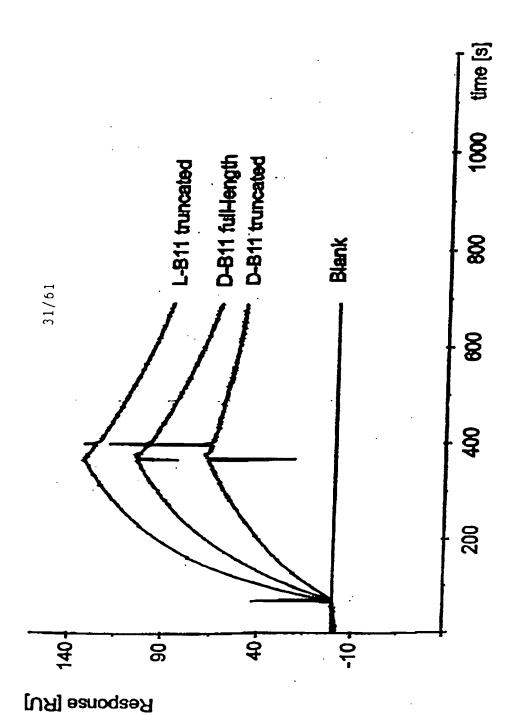


Fig. 2

Sequences derived from the RNA selection (round 13)

32/61

RMA round 13
group1
1.1 main close
'507-R64-D813-85 con

PRINCETCH CONTROL CONTRACTOR CONTRACTOR AND ANTICOMENCE CONTROCT ACCORDING TO A CONTROL CONTROL CONTROL CONTROL CONTROL CONTROCT ACCORDING TO A CONTROL CONTROL CONTROL CONTROL CONTROL CONTROCT ACCORDING TO A CONTROL CONTROL CONTROL CONTROL CONTROL CONTROCT ACCORDING TO A CONTROL CONTRO group2 2.1 meta elema '807-804-EE13-A2

GRADCTCHARCTCHCTCGTGTGTGTGAAGCTAACG-ENAAACCGAAGGTAACCATTCCTACCCCCCGCGTACCAGTGTCCAG

BENGTOLIALCTTCHCTCHTGHOOTALTAUNC-TAUNTCCHAOOTALCCHAC--MOSTACCHCTGTCHAC 3.1 Main close '907-804-D813-CL group3

group4 4.1 main alone 'SOT-R04-DR13-02 concretance

RNA round 14

group1 1.1 (mein close) 'SOT-ROH-IRLE-F7

DAMOCTEMALETTEMETENTIOCOOTUNGOCAAA--COTAACAACOAAAOTAACCATTECTACCAGOTACCACTOTESOFTECAC

variations of clone 1.1

COLOCICLOS CITACOLOS CONTO COCANA — COTANGA COCANA CONTROCIA CONOCIA CON CONTROCIA CON CONTROCIA CON CONTROCIA CON CONTROCIA C - 30T-R04-D014-C11

'SOT-R04-DR14-A8 1.4 'BOT-R04-DR14-C12

GARGOTCHALCTTCALTCATTGOTTAAOGCTAA--COTAAACCAAAGOTAACCATTCCTACCAGGGTACCACTGTCGATTCCAC

group3 3.1 (main eleme) 'SOT-ROM-DRIA-C7

GOLDCTCLOLCTCACTCOTGTGAAGAAAAAAAC---TAAATCCGAAGGTAACCAATCCTALGGTACCAAGTGTCCAC

variations of close 1.1

OCHOCTECHALCITECHATGHOOCHOTANACIT--ANOTECCANOGIMECIATECTALCOTACCACTOTOCACT SOT-R04-DR14-E11

GONOCTICHALCTICALCTICATORIAGOCAATTANACTTG-AAGTCCGAAGGTAACCAATCCTAACGTACCACTGTCGGTTTCCAC '80T-R04-DR14-ED1

GRACTICH BACTT CHAT CON THE TANNAN CIT TOTAN OT COM NOT TANCON THE CITAN CONTINUE TO CONTI SOT-R04-D214-B8

Sequences derived from the 2'F-RNA selection (round 14) '-F-RWA round 14, group1

1.1 (meda clone)

OCHOCTELALCITECHESTS SANIACCIAN TEACTER AND OTITICITICA CANACOTECCOCCOCCOCCOCCOCCACCACCACTICACCACTICACCAC 30T-703-DR14-G6

mutations of close 1.1

OTABET CHARTCH CALT CONTOUND ACT CHARTCH -- CATACONDOCOCOCACCACOTACCACTOR COOTTCCAC * SOT-P03-D&14-P2

GALOCTICAGLICTTICACTICOROGNAZNOGNATICACITECTONOS --- ATNOGNOGICOGOLICOLOGIZACIACOTECCAC 807-P03-DR14-P4.

dalactichalcticalcteotramanamentalancoptiticte-catheoteoecocoleaeorikeeletrosopticela

907-P03-D414-D5

807-P03-D014-G3 907-F03-DR14-B5

ORLOCTECHALCTECH CONTROLATING DANT GALCT CHARCO COLARCO TO GROCCA COLOCAL COLACTA COLOCAL COLOCACIONE COLOCACI

GRACTCLALCTTCLCTCGTGCLATACQLATCACACACOTT.CTC-CALACGTCGCCGCACCLGGTLCCACTGCTACCAC

ORIGETCLASCITCACTCOTOGANTACONATCOTCHARCECTTICTC-CATACOTCCC-- CACCLOGIZACOLCTGCGACTTCCAC

ORLOCTCHALCTTCLCTCGTGGAATAGGAATGGTTTCTT-CATACGTCGCCGCCGCACCLCGTGCCLCTGTCGGTTCCAC oaloctcharctestestestastastastastastas ancottic -- catrostestestestestestestestestes 180T-P03-DR14-C2 - BOT-P03-DR14-P3 SOT-703-DR14-B6 1807-P03-D814-H1

oglociculalcitcacicotographingualtancicamaoriti;----cataboroocogeacopiaccalcitoroori OCHOLOLOLOTTCA.CTCOTOCAATAOCAATGA.CTCACACOT--C---CATACOTCOCCGCA.CCA.CTA.CCA.CTGCOCTTCCA.C ORMOCTICALA CITICAL CITICALA TRACTICA DA COSTITICICA TA COSTICA CO COLO CALCADA CALCADA TACAL CALCADA TACAL C SOT-703-DR14-P6 807-F03-DR14-C1 80T-F03-DR14-B1

Fig. 24

SOT-P03-D014-H5

1.60

OBJECTICABLITICACTION OF THE TOTAL MOCTOCOLOCOT CAPTOCOLOGARA TA COLOCACTOT COLOCACTO BENDOTICHANGITICH.CITEGINGCOCTITICT---MACTETANCCOMCCOTICCORCACOMO--MOSTACCACTOTICHAC <u>ediacticida en transportante de transcontracter de la consconda facena en conscience de la conscience de la</u> GALACTICALCTICALCTOSTICATOTIVACTOCTAACCOTCAATOCOCOCACAAATA.COTACACTOTICAGATTCCA.C OCHACTORICAL CATACOLOGIC CONTRACT - COCHOCOTOLOGICA COLOGICA CATACOLOGICA CATACOLOG OGAOCTICAGACATICACTICOTECCOTTIT - TOTTAGCT - CAGACCOTCAGTOCOGCACGAGATACOTACCAGTOCOGTTICCAC variations of clone 2.1 group2 1.1 (min alone) '807-703-1814-05 '80T-P03-DR14-D1 7.5 '80T-P03-DR14-A2 2.6 '80T-P03-DR14-G2 150T-103-D414-D3 SOT-P03-DX14-H2

1.1 main alone 1807-703-DR14-H6

group3

Fig. 24-2:

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Sequences derived from the 2 F-RNA selection (round 15)

36/61

2'-F-RWA round 15, group1

807-F03-DR15-G10 .. J (main clone)

OGLOCYCLOLACTICALCTOSTOGAATAOGAATGACTCAGACGTTTCT-CATACGT-COCCOCACCACGACGACGACTTOCAC 80T-P03-DR15-G7

GALACTICADACTICALCIOGRANIAGIANICALCICALACTITICI - CALACGT - COCCOCACCACCACOTACCACTIOTICAL GRACT CAGACTICACT COTOGRAPH AGAAT GALCTICA ACOTTIC - CATACTI - COCCOCACCIA COTACCIACTOT COOTTICCAC 'SOT-P03-DR15-P10 SOT-P03-DR15-D9

GRACT CAGACTT CACT COTOGRAATA GRAAT GRACT CRACGOTTT C----ATOT - COCCOCACCA COTACCA CTOTOGRAT CCAC

OGROCTICACTICACTICATICATAGAATAACTICAAAOOTITT---CATACOT-CACCACCAACATAGCACTOTCOGACTICAC OGROCTICACTICACTICACTICATAGAATAACTICAAACOTITT---CATACOT-CACCACCAACATAACCAATTICAAC GOLACT CACACT CACT COT TO GAAL TAGGAAL TACT COACT COACT COCCOCACOL COACT TO COACT TO COACT TO CAC 60T-P03-DR15-P12

SOT-P03-DR15-H7

OCHOCTICACH CACHCOTOGUATHACAIANTGACT CHATHCOT - CATHOOT - CACCICACCACCACCACCACTACCACT TOCOST TOCAC

ORDICTORACTICACTOSTOCANTACIANTECICACIOTITCT-CATACOT-COCCOCACCACOTACCACTOTICCAC OGAGGT CAGACTACA CAGGGAATA OGAAATGA CTTACA GAGGT TACTAC CAGGGCA CCAGACA CAGGGAA CCAGAA CCAGAA CAGAA CCAGAA CCAG SOT-P03-D815-A11 SOT-P03-DR15-PB SOT-P03-D215-A8

BAMBOTICHALTTCH.CTCOTOGOATHACHATICHCHOOCCTTTCTTCHTNAGGT-COCCOCACCALOGTACCACTOTOGOTTCCAC 807-703-DR15-C9

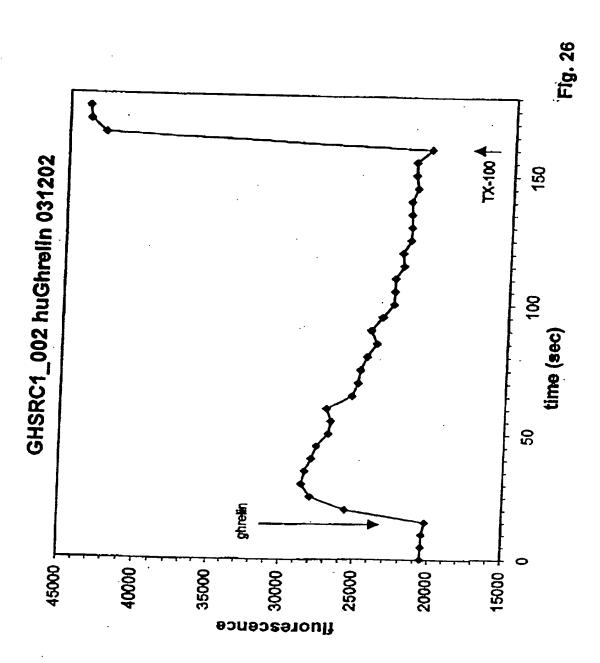
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SOT-703-0015-C12 BOT-F03-DR15-A12 80T-F03-DR15-F7

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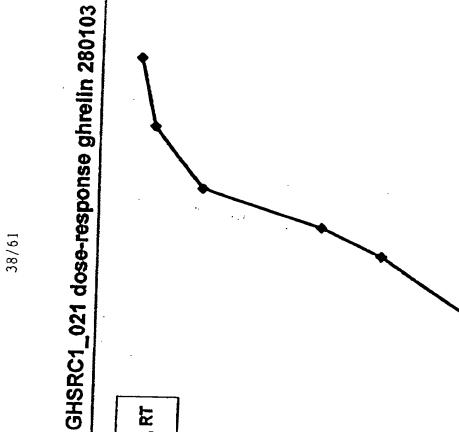
10 10 rat Ghrelin [nM]

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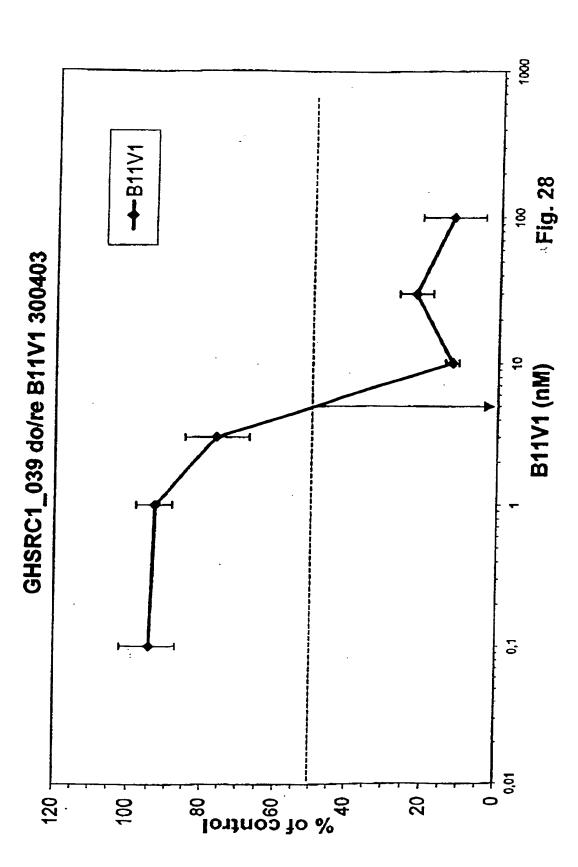
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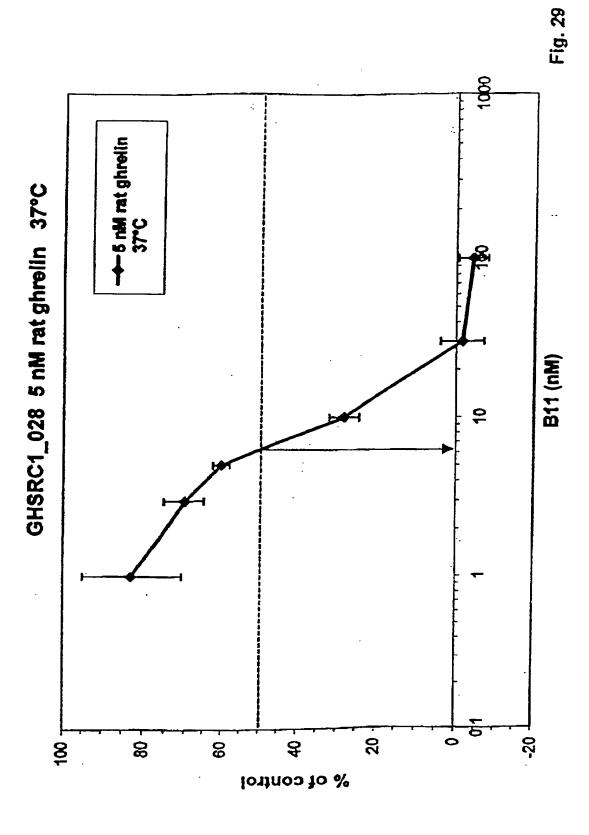
Fmax - Fmin

900

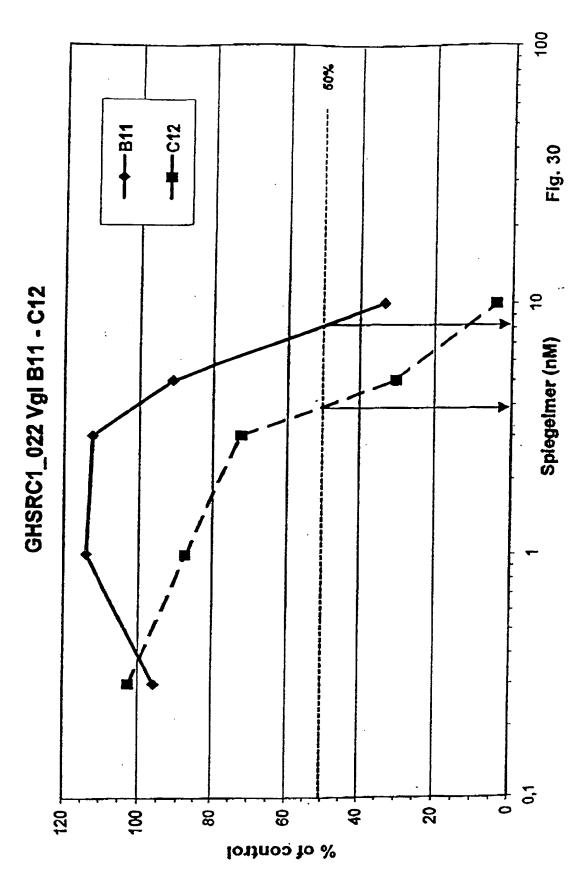
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×- ×-	5'-X-GOTGGGCAGTTATGTAAGACCGAAGGTACCCAATCCTACCCACC-Y-3'	* *		×	* *	×	×	-X-	-X-		-X-	-X-	×	×		-X-	-X-	×	×-	-X	-X-GGTGGGTGAGGCAATCTGGTGAGGCAGATGTAAGACCTAACCTACCCAC-Y-3	STATE TACCEACE -Y-3'
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L01 L02	103	105															21	22	23	24	25	

Pig. 31

Sequence

SEQ ID Identifier

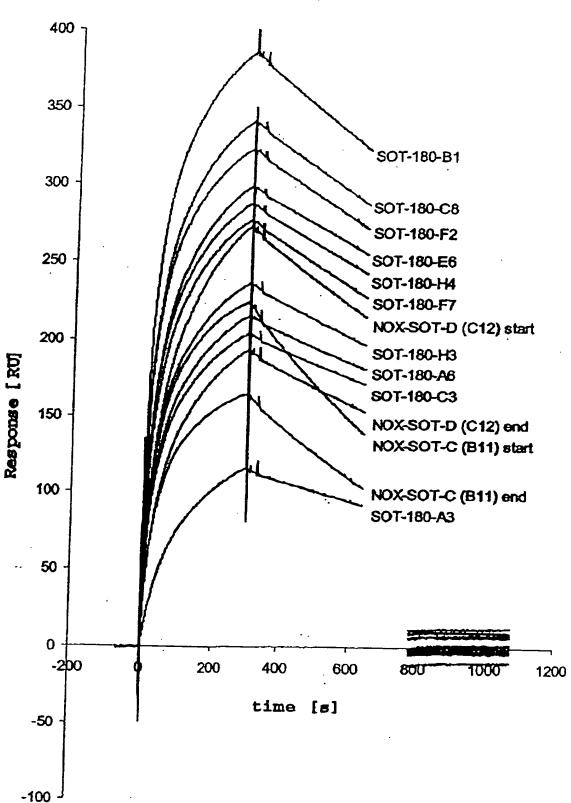


Figure 32A

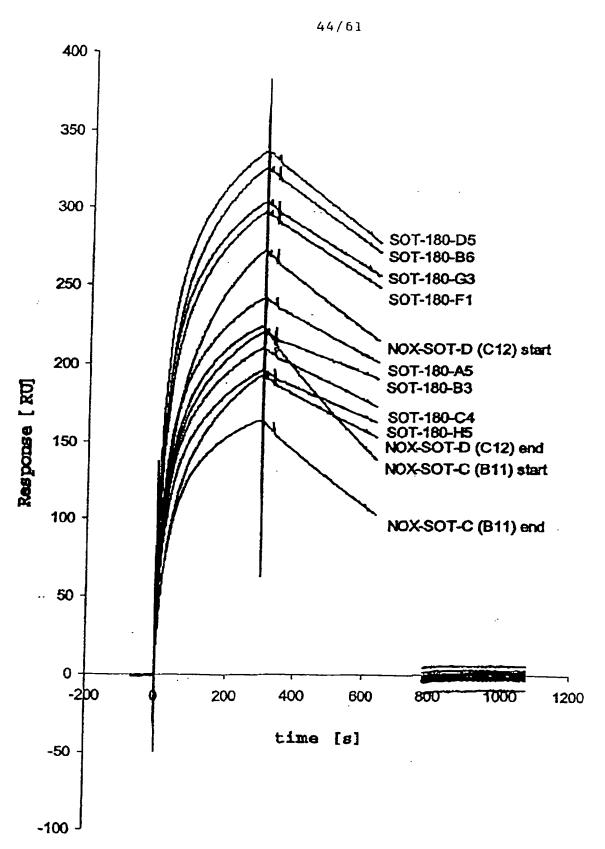


Figure 32B

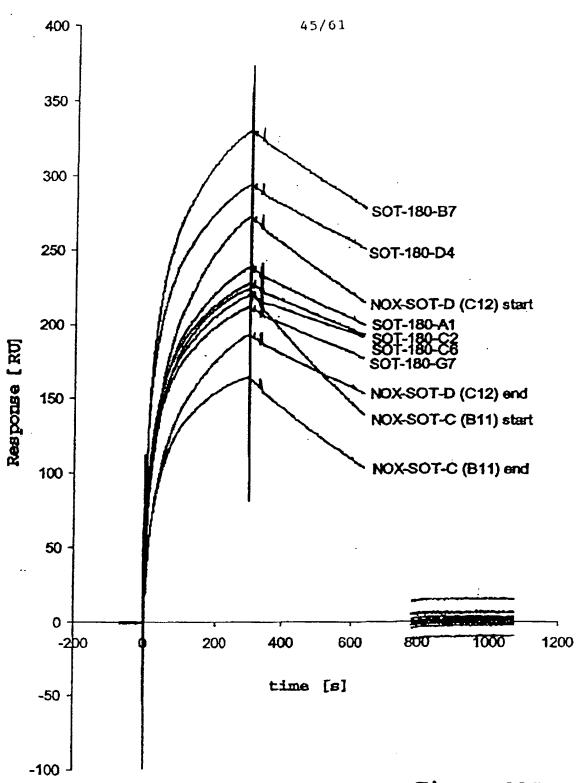


Figure 32C

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96	sot d 1r 057	CONTROLLING CONTRACTOR AND THE CONTRACT AND THE CONTRACT AND CONTRACT	43	SOT-108-C8	
91	d lr		49	SOT-108-F2	
92	sot d 1r 059	GGGTGAGGCANCON ACTIN ACTION ACTIN ACTION ACT	43	SOT-108-F2	
93	Bot d lr 060	GGGTGAGGCAGTTATATATATATATATATATATATATATA	43	SOT-108-B6	
94	sot d lr 061	GGTGGGTGAGGCACACACACACACAGGTACCCAATCCTACCC	43	SOT-108-B7	
95	sot d lr 062	GGGTGAGGCACACCATAAGTCCGAAGGTAACCAATCCTACCCACC	49	SOT-108-D5	
96	sot d lr 063	GGGTGBGGCB ATTOCHMA ACTIONS TO COLOR OF THE	43	SOT-108-D5	
97	got d lr 064	GGGTGAGGCAGTAAAAAAAAAAAAAAAAAAAAAAAAAAA	43	SOT-108-F7	
98	Bot d lr 065	GGGTGB GGCB GTTGA GBB B GTGGA B GGCB A GGCB	43	SOT-108-G3	
66	sot d 1r 066	GGGTGDGGCTBCB AAMA AGGCGT A COMA GGGTA A COMA GGGTGD COMA A COMA	43	SOT-108-H4	
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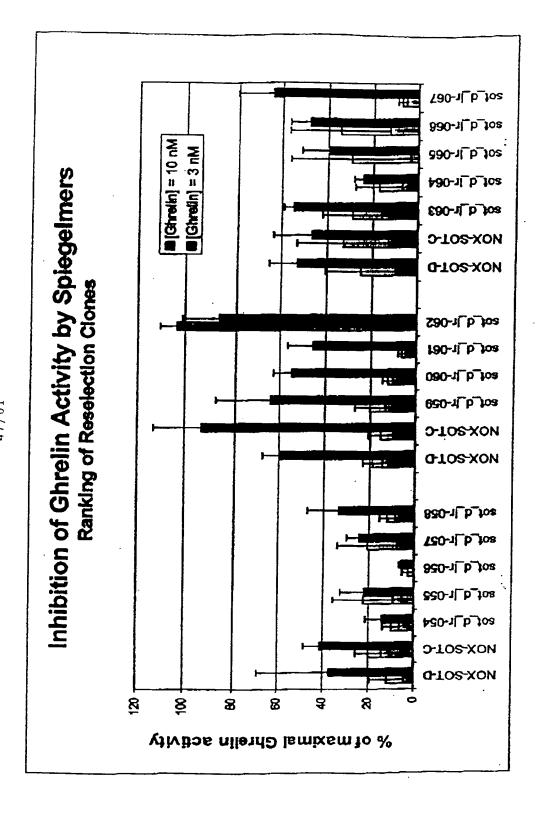
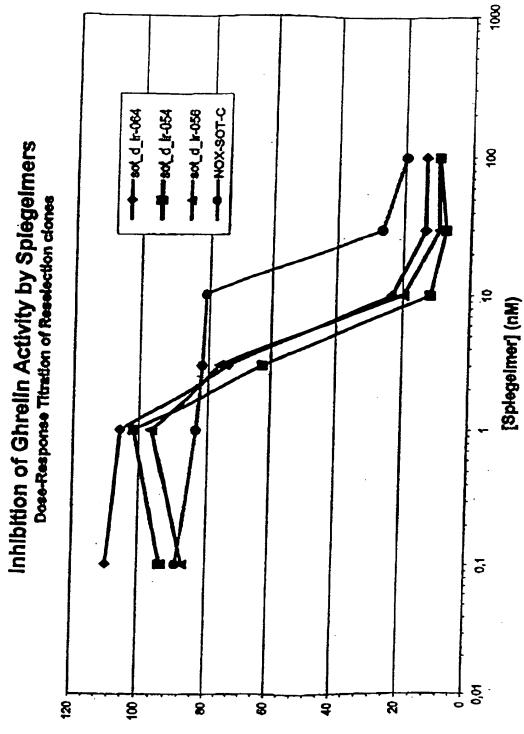


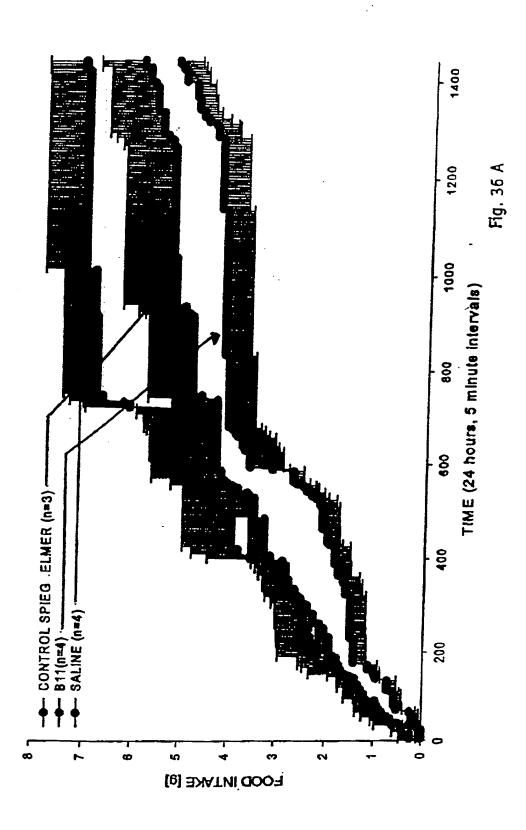
Figure 34

35

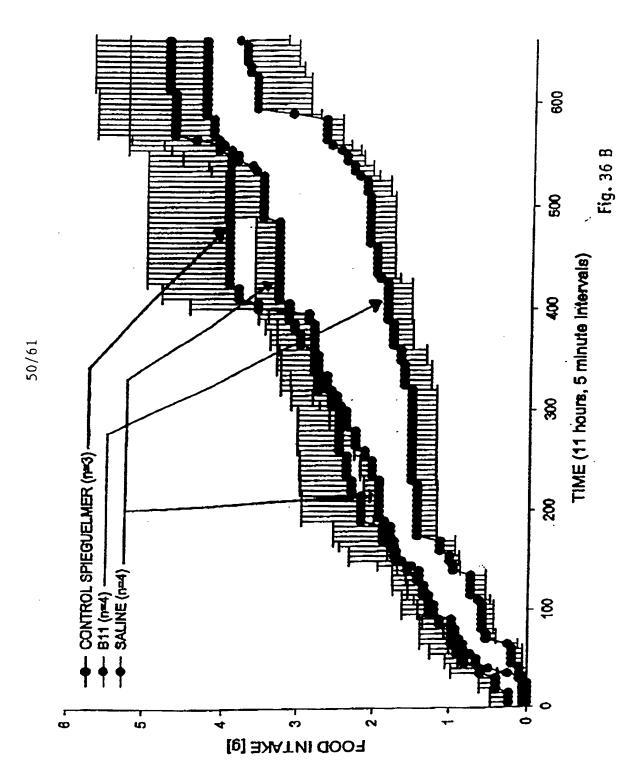
Figure



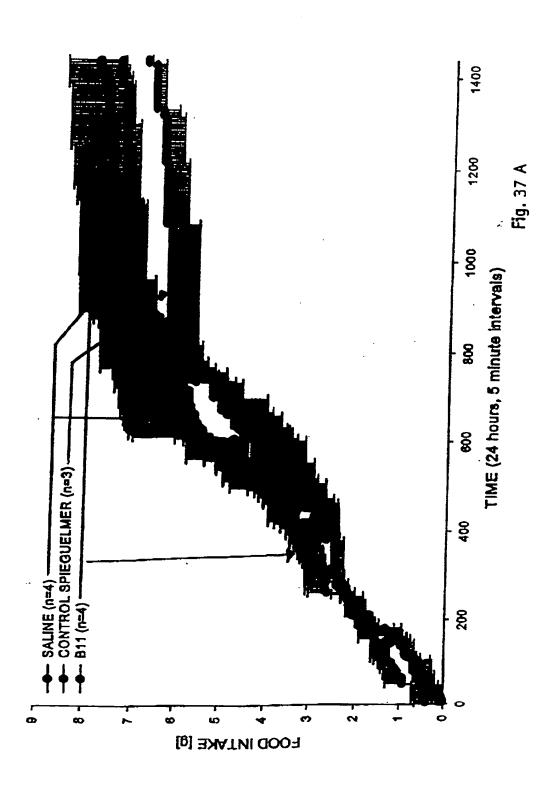
% of maximal Chrelin activity

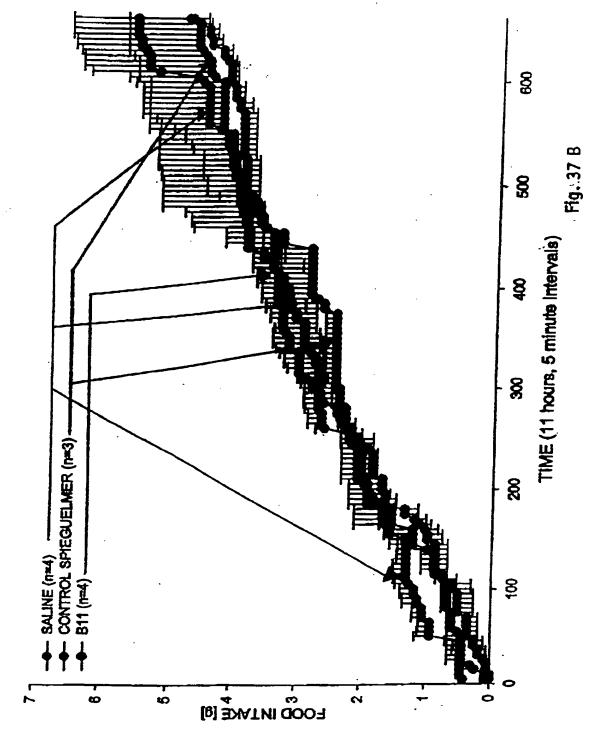


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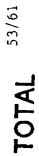


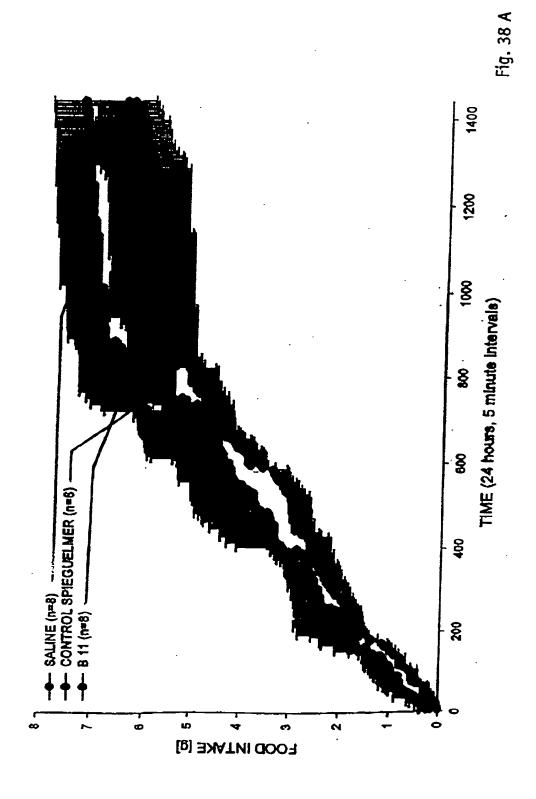
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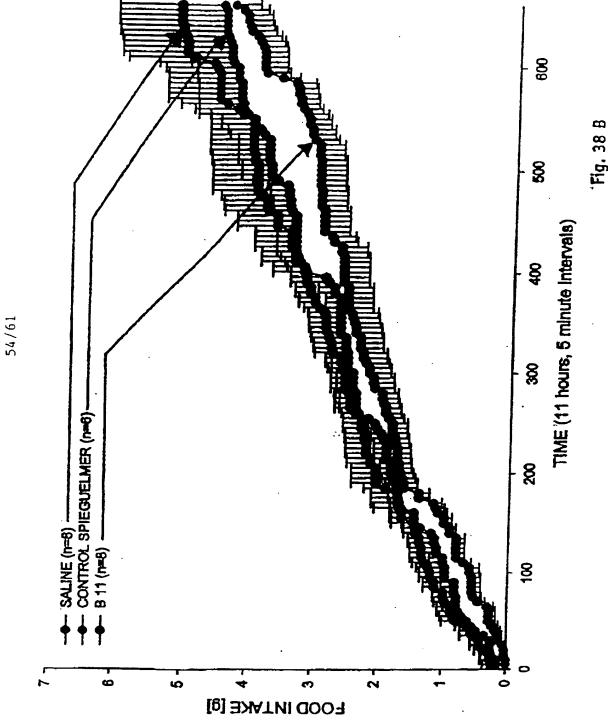




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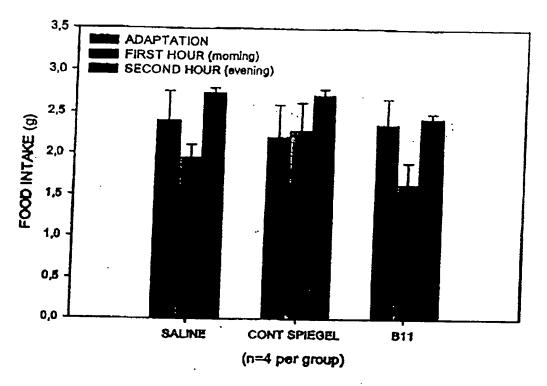




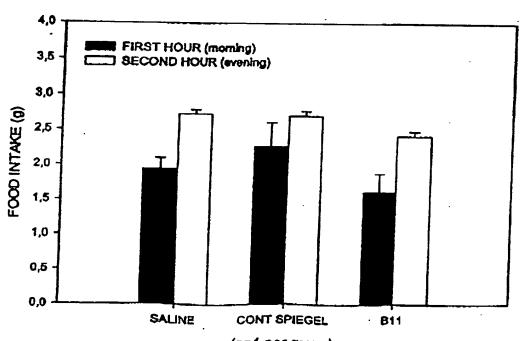
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55/61 MEAL TRAINING FIRST EXPERIMENT



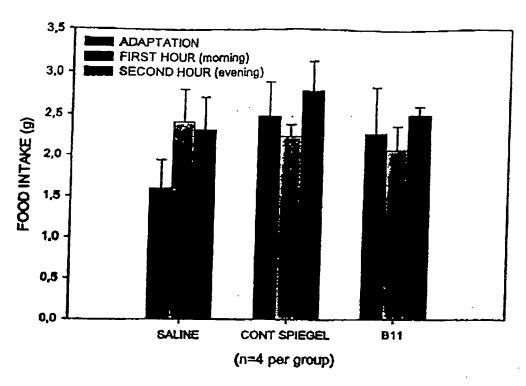
MEAL TRAINING FIRST EXPERIMENT



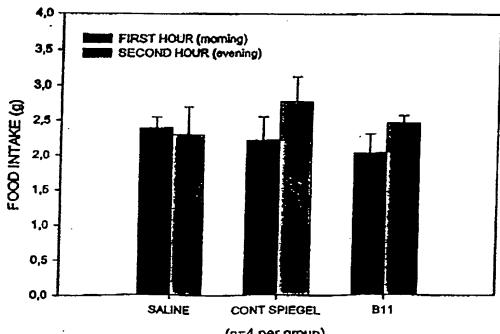
(n=4 per group) Figs. 39 A (top) and 39 B (bottom)

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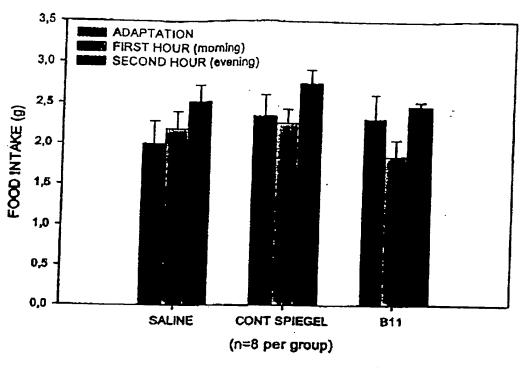
56/61 MEAL TRAINING SECOND EXPERIMENT



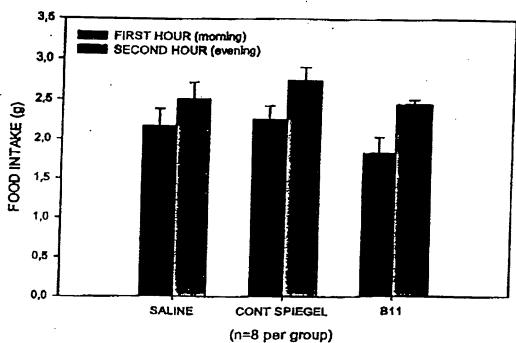
MEAL TRAINING SECOND EXPERIMENT



(n=4 per group)
Figs. 40 A (top) and 40 B (bottom)

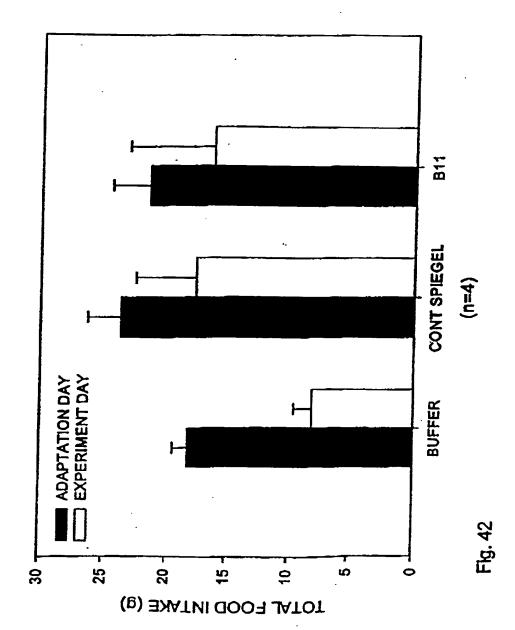


MEAL TRAINING TOTAL

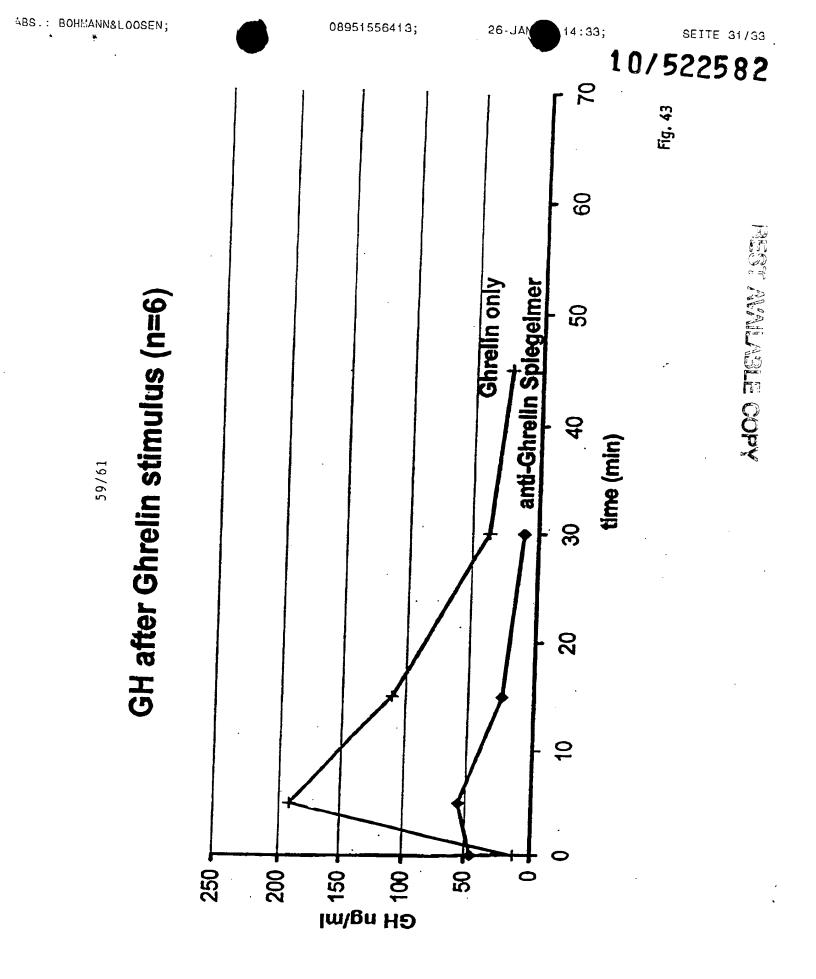


Figs. 41 A (top) and 41 B (bottom)

I.C.V EXPERIMENT (24 hours) (Male Wistar Rats)

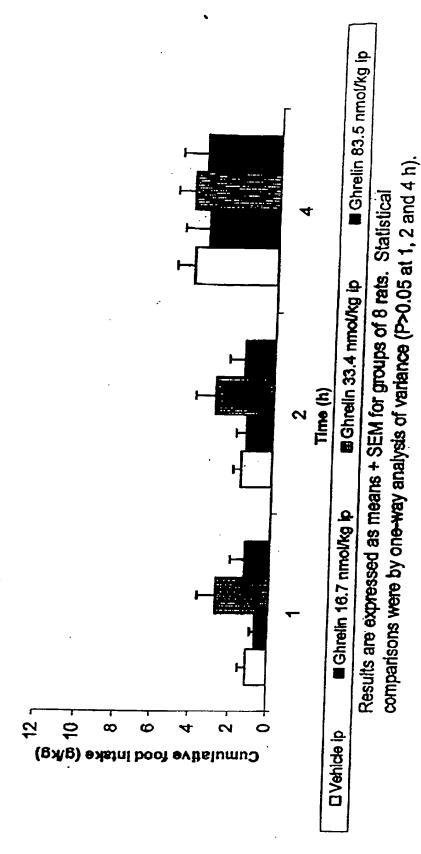


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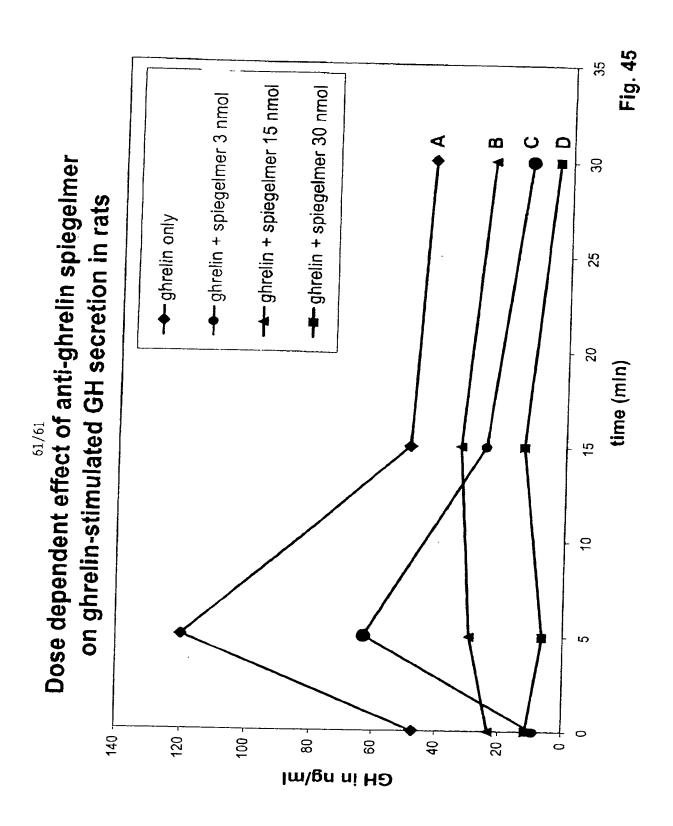




Effects of acute administration of ghrelin on food intake in male Sprague-Dawley rats



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